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Original Research Article

Comparison of the effects of taurine and methionine supplementation on the nitrogen metabolism of beef steers elucidated through plasma metabolome profiling

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ABSTRACT

The objectives of the experiment were to compare the effects of rumen-protected taurine (RPT) and rumen-protected methionine (RPM) on the nitrogen (N) metabolism, plasma biochemical parameters, and metabolomics in beef steers and to clarify whether taurine plays similar roles as methionine (Met) in the regulation of N metabolism in beef steers. Six Simmental steers aged 12 months (liveweight 325 ± 7 kg) were used as experimental animals. The experimental treatments included a basal diet, the basal diet + 70.0 g/d RPT and the basal diet + 74.2 g/d RPM. The treatments were assigned in a replicated 3 × 3 Latin square design. Each experimental period included 15 d for adaptation and 5 d for sampling. The results showed that supplementing the diet with RPT or RPM did not affect the apparent nutrient digestibility ($P > 0.05$). Supplementing the diet with RPT or RPM increased the N retention ($P < 0.05$) and the N utilization efficiency (NUE) ($P < 0.05$) and decreased the urinary excretion of 3-methylhistidine ($P < 0.05$) and the estimated skeletal protein degradation rate ($P < 0.05$). Supplementing the diet with RPT increased the plasma concentrations of taurine ($P < 0.001$), cysteine ($P = 0.010$), valine ($P = 0.013$) and total non-essential amino acids (NEAA) ($P = 0.047$) and tended to increase the plasma concentrations of essential amino acids (EAA) + NEAA ($P = 0.087$), but it did not affect the plasma concentrations of total EAA ($P > 0.05$). Supplementing the diet with RPM increased the plasma concentrations of methionine ($P = 0.033$), lysine ($P = 0.047$), cysteine ($P = 0.007$), leucine ($P = 0.046$), isoleucine ($P = 0.046$), valine ($P = 0.034$), total EAA ($P = 0.028$), total NEAA ($P = 0.004$) and EAA + NEAA ($P = 0.004$). The plasma metabolomics profiling revealed that supplementing the diet with RPT upregulated the plasma concentrations of taurine ($P < 0.001$), L-cysteine ($P = 0.004$) and some amino acid (AA) analogues ($P < 0.05$) and RPM upregulated the plasma concentrations of Met ($P = 0.021$), L-isoleucine ($P = 0.036$), L-tryptophan ($P = 0.006$) and some AA analogues ($P < 0.05$). In conclusion, taurine has similar impacts to Met in improving the N retention and the NUE in beef steers. Taurine deficiency negatively affects the NUE of beef steers. Supplementation of the diet with taurine is beneficial to the N utilization in beef steers.

1. Introduction

Cattle are large animals with high nitrogen (N) intake and high N excretion. The N utilization efficiency (NUE) of beef cattle is only about 23%, which is much lower than other species of domestic animals such as dairy cows (Huhtanen and Hristov, 2009), swine and poultry (Kohn et al., 2005). High N excretion not only increases the protein feed cost but also contaminates the environment. Therefore, improving the NUE and reducing the N excretion are important objectives of the research in beef steer nutrition.

Low protein diets have been found to increase the NUE of ruminants (Knowlton et al., 2010; Batista et al., 2017). However, limiting the N intake also reduces the growth performance and

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productivity of animals (Schiavon et al., 2012). Balancing the dietary amino acid (AA) profile by supplying some essential AA (EAA) can compensate for the performance of animals fed with low N diets to some extent (Wei et al., 2023). Methionine (Met) is an important sulfur-containing AA, which is considered the first limiting AA in beef steers (Klemesrud et al., 2000). Absorbed Met can be used directly for protein synthesis and S-adenosylmethionine production in animals (Hernández et al., 2017) and can be also used for taurine synthesis in the liver of animals (Wu, 2013). Taurine is a non-proteinogenic AA and is the most abundant sulfur-containing free AA in the animal body (Huxtable, 1992), and it plays many important roles in physiology and nutritional metabolism (Wu, 2020).

Although taurine can be synthesized endogenously mainly in the liver in most mammals, the amount of self-synthesized taurine in animals is insufficient to meet their requirements for taurine (García-Ayuso et al., 2024). Feeds of animal-origin contain varying levels of taurine whereas feeds of plant-origin hardly contain any taurine (Laidlaw et al., 1990; Sturman, 1993). Since beef steers are herbivores and their diets contain no feeds of animal origin, they may be in the status of taurine deficiency. Hence, supplementing with taurine not only meets the taurine requirement of beef steers, but also reduces the amount of Met converted to taurine, and more Met can be used for body protein synthesis in the animals. Recent studies showed that dietary supplementation of unprotected taurine increased the rumen microbial crude protein (MCP) synthesis but it did not affect the N retention and the NUE in beef steers (Liu et al., 2023), possibly because taurine was highly hydrolyzed in the rumen as reported in an in vitro rumen fermentation study (Zhang et al., 2023). The objectives of this experiment were to compare the effects of dietary supplementation with rumen-protected taurine (RPT) and rumen-protected methionine (RPM) on the N retention (NR) and the NUE in beef steers, and clarify if taurine deficiency is an important reason for the low NUE of beef cattle and further elucidate the mechanisms through plasma metabolomics profiling.

2. Materials and methods

2.1. Animal ethics statement

The feeding and management of animals were approved by the Laboratory Animal Welfare and Animal Experimental Ethical Inspection of China Agricultural University (AW31213202-1-1).

2.2. Animals and experimental design

Six Simmental steers aged 12 months with 325 ± 7 kg of initial body weight were used for the experiment. RPT and RPM were used to supply taurine and Met to beef steers because taurine and Met are highly hydrolyzable in rumen fermentation (Zhang et al., 2023).

Taurine (purity 99.0%) and Met (DL-Met, purity 99.0%) were purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. (Shanghai, China) and were rumen-protected by King Techina (Hangzhou, Zhejiang, China) using corn starch and palm oil as the protection materials. The RPT contained 60% of taurine with 87.34% in vitro rumen-bypass rate and 82.96% of in vitro digestibility and the RPM contained 60% of Met with 83.96% in vitro rumen-bypass rate and 81.69% in vitro digestibility, respectively. The values of in vitro rumen-bypass rate and digestibility of RPT and RPM were determined using the two-step digestion technique of Tilley and Terry (1963).

The treatment groups included a basal diet (control group), the basal diet + 70.0 g/d RPT and the basal diet + 74.2 g/d RPM (Table 1). Based on the content of taurine in RPT or Met in RPM and the *in vitro* rumen-bypass rate and the *in vitro* digestibility of RPT or RPM, supplementation with 70.0 g/d RPT or 74.2 g/d RPM would release about 30.5 g taurine or Met in the small intestine of steers daily. The steers and treatment groups were assigned in a replicated 3 × 3 Latin Square design. Each period included a 15-day adaptation phase and a 5-day sampling phase. The steers were on restricted feeding and each steer was fed with a total mixed ration (TMR) containing 3.16 kg corn silage (dry matter, DM) and 2.45 kg concentrate mixture per day, which supplied about 90% of the ad libitum DM intake of the animals. The daily TMR and the designed amount of RPT or RPM were divided into two equal parts and were supplied to each animal at 07:00 and 17:00, respectively. The RPM and RPT were completely mixed with TMR before feeding. The steers had free access to fresh drinking water. The live weight of each beef steer was recorded on the first and last day of each experimental period before morning feeding.

Table 1
Ingredients and nutritional composition of the basal diet (DM basis, %).

Item	Content
Ingredients	
Corn silage	55.00
Corn grain	26.50
Soybean meal	10.46
Wheat bran	7.34
Sodium bicarbonate	0.35
Salt	0.35
Total	100.00
Nutritional composition	
OM	94.17
CP	12.26
NDF	48.93
ADF	26.63
GE, MJ/kg	16.11
NE_{mf}, MJ/kg[1]	4.63
3-Methyl-histidine, μmol/g	1.909

DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber; GE = gross energy; NE_{mf} = net energy for maintenance and fattening.
[1] Calculated based on the dietary GE, OM, and NDF contents according to the Nutrient Requirements and Feeding Standards of Beef Cattle (Feng, 2000).

2.3. Sampling

The experimental animals were tethered in individual pens (1.5 m × 2.5 m) with mats. During the sampling period, feces were collected from each steer on a daily basis using a bucket placed behind each animal, and the weight of feces was recorded. After homogenization, 1% of the feces from each steer was sampled and added with 20 mL H_2SO_4 (10%, v/v) to avoid the N loss. During the sampling period, the urine was also collected from each steer on a daily basis, using a funnel connected with a polyethylene pipe to a plastic bucket surrounded by ice cubes. The daily total urine output from each steer was recorded and homogenized. An aliquot of 70 mL urine from each steer was sampled and added with 10 mL H_2SO_4 (10%, v/v) for preserving the N. Corn silage and concentrate mixtures were also sampled daily.

On the first day of each sampling period before morning feeding, a volume of about 20 mL of blood was taken from the jugular vein of each steer into a tube containing heparin (Shandong Osat Medical Device Co., Ltd., Shandong, China). The plasma samples were obtained by centrifuging the blood samples at 3000 × *g* for 15 min at 4 °C. On the same day of each sampling period 3 h after morning feeding, the rumen fluid was taken from each steer via the esophagus of each steer using a stomach tube. The first tube of rumen fluid was discarded to avoid saliva

contamination and the second tube of rumen fluid of about 100 mL was kept as the sample. The pH values of the rumen fluid samples were immediately measured using a portable pH meter (model 8601, AZ Instruments Co. Ltd., Guangdong, China) and then filtered through four layers of surgical gauze. All samples were stored in a freezer at −20 °C.

2.4. Chemical analyses

The corn silage and fecal samples were freeze-dried for 72 h using a freeze dryer (LGJ-12; Beijing Songyuan Huaxing Technology Development Co., Ltd., Beijing, China). The freeze-dried feed samples were milled and the fecal samples were ground using a mortar and a pestle to pass a sieve with 1 mm pore size. The DM and crude ash of feeds and feces were analyzed according to AOAC (2005) using methods 930.15 and 942.05, respectively. The organic matter (OM) of feeds and feces was calculated by DM minus crude ash. The N contents of feeds, urine and feces were analyzed by the Kjeldahl method according to AOAC (2005) using method 984.10. The crude protein (CP) contents of feeds and feces were calculated by total N × 6.25. The neutral detergent fiber (NDF) was analyzed using the procedures of Van Soest et al. (1991) with heat-stable α-amylase and sodium sulfite (Mertens, 2002). The acid detergent fiber (ADF) of feeds and feces was analyzed according to AOAC (2005) using method 973.18. Both NDF and ADF were analyzed on an Ankom A200i fiber analyzer (Ankom Technology Co., NY, USA). The gross energy (GE) of feeds was analyzed on an oxygen bomb calorimeter (Parr 6300 Calorimeter; Parr Instrument Company, Moline, IL, USA).

The ruminal ammonia nitrogen (NH_3—N) was analyzed using the colorimetric method of Broderick and Kang (1980) on a spectrophotometer (UV-1801; Beijing Rayleigh Analytical Instrument Co. Ltd., Beijing, China). The volatile fatty acids (VFA) of rumen fluid were analyzed on a gas chromatograph (GC-8600; Beijing Beifen Tianpu Instrument Technology Co., Ltd., Beijing, China) using the method described by Yang et al. (2017). The rumen MCP was determined using the method of Makkar et al. (1982).

The plasma total protein (TP), albumin (ALB), globulin (GLB), triglyceride (TG), urea (UREA), glucose (GLU), total antioxidant capacity (T-AOC), growth hormone (GH) and insulin-like growth factor-1 (IGF-1) were analyzed using analytical kits (Beijing Sino-UK Institute of Biological Technology, Beijing, China) on an automatic biochemical analyzer (7170; Hitachi Ltd., Tokyo, Japan).

The urea and creatinine contents in urine samples were analyzed on a spectrophotometer (UV-1801; Beijing Rayleigh Analytical Instrument Co., Ltd.) using commercial kits (Nanjing Jiancheng Bioengineering Institute, Jiangsu, China) based on the diacetyl monoxime method and Jaffe's assay, respectively. The hippuric acid of urine samples was analyzed according to the procedures of China Hygienic Standard (WS/T 52-1996) based on the chemical reaction of hippuric acid with benzene sulfonyl chloride in the presence of quinoline to form yellow compounds and the colorimetric determinations of the color depth of the liquids on a spectrophotometer (UV-1801; Beijing Rayleigh Analytical Instrument Co. Ltd.). The urinary allantoin and uric acid were analyzed using the methods of Chen and Gomes (1992). The taurine in urine was analyzed on HPLC (LC98-1; Wenfen Analytical Instrument Technology Development Co. Ltd., Beijing, China.) equipped with a HyperClone BDS C_{18} column (250 mm × 4.6 mm, 5 μm) according to Huang et al. (2016). The 3-methyl-histidine (3-MeHis) in urine and feeds were analyzed on a microplate reader (DR-200BS; Wuxi Hiwell-Diatek Instruments Co. Ltd., Jiangsu, China) using analytical kits (Beijing Sino-UK Institute of Biological Technology).

The AA of plasma samples were analyzed using HPLC (Ultimate 3000, USA)-tandem mass spectrometry (API 3200 Q-TRAP, USA) (HPLC-MS/MS) using the method described by Shimbo et al. (2010).

The plasma metabolomics were analyzed using an LC-MS/MS system (UHPLC-Q Exactive HF-X; Thermo, USA) equipped with an HSS T3 column (100 mm × 2.1 mm i.d., 1.8 μm; Waters, USA).

2.5. Calculations

The NR and NUE were calculated as:

$$\text{NR} = \text{nutrient intake} - \text{fecal N} - \text{urinary N};$$
$$\text{NUE} = 100 \times \text{NR}/\text{N intake}$$

where the unit for NR, nutrient intake, fecal N and urinary N is g/d; the unit for NUE is %.

The rumen microbial N supply was estimated according to Chen and Gomes (1992):

$$Y = 0.85X + \left(0.385 \times \text{BW}^{0.75}\right)$$

where Y is the total urinary purine derivatives (PD; uric acid + allantoin), mmol/d; X is the absorbed PD excretion, mmol/d; $\text{BW}^{0.75}$ is the metabolic liveweight of a steer, kg; 0.85 is the recovery rate of absorbed purines as PD in urine; 0.385 is the endogenous excretion of PD, mmol/kg $\text{BW}^{0.75}$ per day.

$$\text{MN} = (X \times 70)/(0.116 \times 0.83 \times 1000)$$

where MN is the microbial N supply, g/d; X is the absorbed PD excretion, mmol/d; 70 is the N content of purines, mg N/mmol; 0.116 is the ratio of purine N to total N of mixed rumen microbes; 0.83 is the digestibility of microbial purines.

The skeletal muscle protein degradation rate (SMPD) was calculated according to Funabiki et al. (1976), Nishizawa et al. (1979) and He and Lin (2000):

$$Y = 0.934 \times (E - 0.8 \times \text{F})/P$$

where Y is the skeletal muscle protein degradation rate, %; E is the urinary excretion of 3-MeHis, μmol/d; F is the dietary intake of 3-MeHis, μmol/d; P is the storage of 3-MeHis in the body of each steer, μmol; 0.934 is the ratio of 3-MeHis in skeletal muscle to the body reserve; 0.8 is the recovery rate of ingested 3-MeHis from diets.

2.6. Statistical analysis

The experimental data were analyzed by the mixed linear model of SAS 9.4 (SAS Inst. Inc., Cary, NC, USA) using the model as follows:

$$Y_{ijkl} = \mu + T_i + P_j + S_k + C_l + e_{ijkl}$$

where Y_{ijkl} is the dependent variable; μ is the overall mean; T_i is the effect of treatment; P_j is the random effect of period; S_k is the random effect of square; C_l is the random effect of animals; e_{ijkl} is the error residual.

The contrast statement was used for comparing the differences among treatments (control vs RPT; control vs RPM; RPT vs RPM). $P \leq 0.05$ represents a significant difference, $0.05 < P < 0.10$ represents a trend.

The ropls (Version 1.6.2) of the R package were used to perform the multivariate statistical analysis. The principle component analysis (PCA) was applied to obtain an overview of the metabolic data, general clustering, trends, or outliers. The metabolite

Table 2
Effects of dietary supplementation with RPT and RPM on the rumen fermentation parameters in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
pH	6.66	6.66	6.69	0.023	0.960	0.347	0.323
NH_3–N, mg/100 mL	7.63	7.79	7.41	0.486	0.818	0.749	0.584
Total VFA, mmol/L	83.80	86.22	83.60	6.188	0.786	0.982	0.769
Molar percentages of total VFA, mmol/100 mmol							
Acetate	61.91	60.97	60.60	0.449	0.160	0.057	0.570
Propionate	19.12	20.52	20.08	0.656	0.151	0.318	0.638
Isobutyrate	1.56	1.46	1.62	0.075	0.383	0.586	0.166
Butyrate	14.31	13.65	14.34	0.594	0.447	0.974	0.428
Isovalerate	2.12	2.34	2.37	0.156	0.327	0.268	0.893
Valerate	0.99	1.05	1.00	0.052	0.407	0.904	0.477
Acetate/propionate	3.26	2.99	3.03	0.114	0.108	0.171	0.791
MCP, mg/mL	0.14	0.17	0.17	0.028	0.519	0.576	0.930

RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; NH_3–N = ammonia nitrogen; VFA = total volatile fatty acids; MCP = microbial crude protein.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM. Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.

variables were scaled to unit-variances prior to conducting the PCA. The orthogonal partial least squares discriminate analysis (PLS-DA) was used to determine the global metabolic changes between groups. The metabolite variables were scaled to Pareto scaling prior to conducting the PLS-DA. The model validity was evaluated from model parameters R2 and Q2, which provided interpretability and predictability, respectively. The variable importance in the projection (VIP) was analyzed using the OPLS-DA model. The fold change (FC) value of each metabolite was calculated by comparing the mean values of the peak area of each metabolite of all samples within the same group, and the FC value was used to indicate the specific variable quantity in the comparison. The *P*-values were estimated with paired *t*-test on single dimensional statistical analysis. The differential metabolites were used to perform the metabolic pathway analysis using MetaboAnalyst 4.0.

3. Results

3.1. Rumen fermentation parameters

No significant differences were found among the three groups in ruminal pH, concentrations of NH_3–N, MCP and total VFA, and the molar proportions of individual VFA and the acetate/propionate ratio ($P > 0.05$) (Table 2).

3.2. Apparent nutrient digestibility

No significant differences were found among the three groups in the apparent digestibility of DM, OM, CP, NDF and ADF ($P > 0.05$) (Table 3).

3.3. N balance, urinary nitrogenous compounds and average daily gain

Table 4 indicates that adding RPT decreased the urinary N excretion ($P = 0.007$), and increased the NR ($P = 0.004$), the NUE ($P = 0.008$) and the average daily gain (ADG) ($P = 0.006$) compared with the control group. Supplementation with RPM decreased the urinary N excretion ($P = 0.006$) and increased the NR ($P = 0.002$), the NUE ($P = 0.003$) and the ADG ($P = 0.006$) compared with the control group. No differences were found between the RPT group and RPM group in these indices ($P > 0.05$).

Table 5 shows that adding RPT decreased the urinary excretion of urea ($P = 0.047$) and increased the urinary excretions of hippuric acid ($P = 0.010$) and taurine ($P < 0.001$) compared with the control group. Adding RPM decreased the urinary excretion of urea ($P = 0.046$) and increased the urinary excretion of hippuric acid ($P = 0.006$) compared with the control group (Table 5), but it did not affect the urinary excretion of taurine ($P = 0.108$). Adding RPT increased the urinary excretion of taurine compared with RPM ($P = 0.002$). No significant differences were found among the groups in the urinary excretions of allantoin, uric acid and creatinine ($P > 0.05$).

3.4. Urinary excretion of 3-MeHis and skeletal muscle protein degradation rate

Table 5 indicates that RPT supplementation decreased the urinary excretion of 3-MeHis ($P = 0.022$) and the estimated skeletal muscle protein degradation rate ($P = 0.014$) compared with the control group. RPM supplementation decreased the urinary

Table 3
Effects of dietary supplementation with RPT and RPM on the apparent nutrient digestibility (%) in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
DM	67.80	71.63	71.26	2.195	0.238	0.284	0.907
OM	67.93	71.78	71.49	2.219	0.239	0.275	0.926
CP	66.88	67.26	68.72	1.018	0.791	0.220	0.328
NDF	55.38	58.83	58.47	1.978	0.236	0.286	0.900
ADF	48.52	50.44	52.35	1.577	0.403	0.106	0.404

RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; DM = dry matter; OM = organic matter; CP = crude protein; NDF = neutral detergent fiber; ADF = acid detergent fiber.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM., Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.

Table 4
Effects of dietary supplementation with RPT and RPM on the N balance and ADG in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
DMI, kg/d	5.61	5.61	5.61	–	–	–	–
Intake of RPT or RPM, kg/d	0.00	0.070	0.074	–	–	–	–
N of RPT or RPM, g/d	0.00	4.70	4.18	–	–	–	–
N intake, g/d	110.12	114.82	114.30	–	–	–	–
Fecal N, g/d	36.47	38.03	36.20	1.156	0.355	0.872	0.281
Urinary N, g/d	51.33	42.47	42.43	1.986	0.007	0.006	0.989
NR, g/d	22.31	34.32	35.67	2.511	0.004	0.002	0.710
NUE, %	20.27	29.89	31.20	2.217	0.008	0.003	0.681
ADG, kg/d	0.72	1.23	1.23	0.113	0.006	0.006	0.959

RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; DMI = dry matter intake; N = nitrogen; NR = nitrogen retention; NUE = nitrogen utilization efficiency; ADG = average daily gain.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM., Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.

Table 5
Effects of dietary supplementation with RPT and RPM on the urinary nitrogenous compounds, 3-methylhistidine and skeletal muscle protein degradation rate in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
Urinary urea, mol/d	1.35	1.12	1.11	0.076	0.047	0.046	0.993
Allantoin, mmol/d	102.24	97.84	99.36	5.392	0.572	0.711	0.844
Uric acid, mmol/d	4.45	3.82	4.28	0.562	0.436	0.830	0.569
Creatinine, mmol/d	91.42	92.84	90.15	3.771	0.794	0.815	0.622
Hippuric acid, mmol/d	130.37	147.52	149.14	4.100	0.010	0.006	0.785
PD, mmol/d	106.70	101.66	103.65	5.569	0.532	0.704	0.804
MN, g/d	55.86	51.82	53.23	4.029	0.490	0.652	0.808
3-Methylhistidine, mmol/d	3.03	2.70	2.69	0.091	0.022	0.018	0.909
SMPD, %	3.59	3.15	3.10	0.113	0.014	0.008	0.789
Taurine, g/d	0.16	0.43	0.24	0.035	< 0.001	0.108	0.002

RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; PD = purine derivatives; MN = microbial nitrogen; SMPD = skeletal muscle protein degradation rate.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM., Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.

excretion of 3-MeHis ($P = 0.018$) and the estimated skeletal muscle protein degradation rate ($P = 0.008$) compared with the control group. No differences were found between RPT and RPM groups in the urinary excretion of 3-MeHis ($P = 0.909$) and the estimated skeletal muscle protein degradation rate ($P = 0.789$).

3.5. Plasma biochemical parameters

No differences were found among the three groups in plasma concentrations of TP, ALB, GLB, TG, UREA, GLU, T-AOC, IGF-1 and GH ($P > 0.05$) (Table 6).

3.6. Plasma AA

Table 7 shows that supplementing with RPT increased the plasma concentrations of taurine ($P < 0.001$), cysteine ($P = 0.010$), valine ($P = 0.013$), serine ($P = 0.025$), glycine ($P = 0.030$), alanine ($P = 0.045$) and total NEAA ($P = 0.047$), and tended to increase the plasma concentration of the total AA ($P = 0.087$) compared with the control group. Supplementation with RPM increased the plasma concentrations of Met ($P = 0.033$), cysteine ($P = 0.007$), lysine ($P = 0.047$), valine ($P = 0.034$), isoleucine ($P = 0.046$), leucine ($P = 0.046$), asparagine ($P = 0.014$), glycine ($P = 0.001$), alanine ($P = 0.010$), total EAA ($P = 0.028$), total NEAA ($P = 0.004$) and the total AA ($P = 0.004$) compared with the control group. Table 7 also shows that RPT supplementation increased the plasma concentration of taurine ($P < 0.001$) and serine ($P = 0.040$), but decreased Met ($P = 0.006$), and tended to decrease glycine ($P = 0.079$) compared to RPM supplementation.

3.7. Plasma metabolome

3.7.1. Plasma metabolites

The LC-MS analysis indicated that a total of 7585 peaks of the mass spectrum were obtained, of which 1185 metabolites were detected and 694 metabolites were identified based on the KEGG database. The PCA score plot revealed that the variations of the first and the second principal components were 10.80% and 34.90%, respectively (Fig. S1). The PCA analysis showed that no difference was found in the plasma metabolites among the three groups. The PLS-DA analysis showed that the separation among groups was clear and all samples were in the 95% confidence circle (Fig. S2). The permutation test indicated that the range of R2 values was appropriate, and the Q2 intercept among the three groups was <0 and R2Y (cum) > 0.9, suggesting that the model was valid and not overfitting, which could be used to test for the differences among three groups (Fig. S3).

The VIP values which were calculated by the OPLS-DA model and combined with statistical analysis showed that there were 98 differential metabolites between the RPT and the control groups, 43 differential metabolites between the RPMand the control groups, and 143 differential metabolites between the RPT and the RPM groups ($P < 0.05$ and VIP >1). Table 8 shows that compared with the control group, supplementation with RPT increased the plasma

Table 6
Effects of dietary supplementation with RPT and RPM on the plasma metabolites in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
TP, g/L	67.78	69.07	66.76	1.132	0.430	0.537	0.170
ALB, g/L	30.91	30.68	30.66	0.785	0.835	0.822	0.986
GLB, g/L	36.86	38.40	36.11	1.122	0.349	0.640	0.169
TG, mmol/L	0.17	0.20	0.19	0.017	0.392	0.516	0.831
UREA, mmol/L	2.46	2.62	2.64	0.183	0.550	0.491	0.927
GLU, mmol/L	5.25	5.33	5.32	0.117	0.626	0.663	0.959
T-AOC, U/mL	11.72	11.51	11.46	0.554	0.787	0.745	0.956
IGF-1, ng/mL	191.85	199.58	189.04	13.671	0.695	0.887	0.594
GH, ng/mL	5.35	4.31	4.76	0.425	0.105	0.345	0.466

RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; TP = total protein; ALB = albumin; GLB = globulin; TG = triglyceride; UREA = urea; GLU = glucose; T-AOC = total antioxidant capacity; IGF-1 = insulin-like growth factor-1; GH = growth hormone.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM., Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.

Table 7
Effects of dietary supplementation with RPT and RPM on the plasma AA (μmol/L) in beef steers.[1]

Item	Control	RPT	RPM	SEM	*P*-value		
					Control vs RPT	Control vs RPM	RPT vs RPM
Taurine	8.04	19.32	9.36	1.315	< 0.001	0.490	< 0.001
3-Methyl-histidine	8.00	8.17	7.58	0.893	0.894	0.745	0.647
Cysteine	0.46	0.91	1.29	0.189	0.010	0.007	0.178
EAA							
Methionine	16.07	14.37	20.80	1.429	0.413	0.033	0.006
Lysine	61.98	68.03	85.38	7.654	0.584	0.047	0.130
Phenylalanine	31.72	31.37	32.50	1.743	0.889	0.755	0.652
Tryptophan	24.95	23.62	24.95	1.105	0.407	0.995	0.407
Valine	132.00	147.83	145.00	3.951	0.013	0.034	0.620
Threonine	47.68	43.42	49.47	5.339	0.580	0.817	0.435
Isoleucine	59.10	64.47	66.75	2.484	0.147	0.046	0.526
Leucine	78.22	83.58	87.25	2.936	0.216	0.046	0.391
Histidine	42.37	42.00	50.68	4.378	0.954	0.199	0.181
Tyrosine	23.73	24.80	26.73	1,480	0.618	0.172	0.370
Total EAA	517.82	543.48	589.52	20.771	0.396	0.028	0.138
NEAA							
Asparagine	49.13	55.00	62.40	3.392	0.240	0.014	0.143
Serine	71.77	85.95	73.17	4.025	0.025	0.809	0.040
Aspartic acid	2.67	2.67	3.50	0.440	1.000	0.201	0.201
Glutamine	218.33	225.17	231.67	9.624	0.623	0.343	0.640
Glutamic acid	58.57	60.43	60.13	5.616	0.817	0.846	0.970
Glycine	285.67	317.83	343.17	9.486	0.030	0.001	0.079
Alanine	205.33	225.50	232.50	6.505	0.045	0.010	0.459
Arginine	66.62	61.87	67.40	4.912	0.505	0.912	0.438
Proline	55.88	56.72	59.73	2.445	0.813	0.283	0.397
Total NEAA	1013.97	1091.13	1133.67	25.173	0.047	0.004	0.251
Total AA[2]	1531.78	1634.62	1723.18	39.658	0.087	0.004	0.135

AA = amino acids; RPT = rumen-protected taurine; RPM = rumen-protected methionine; SEM = standard error of the mean; EAA = essential amino acids; NEAA = non-essential amino acids.
[1] Contral, basal diet; RPT, basal diet + 70.0 g/d RPT; RPM, basal diet + 74.2 g/d RPM., Differences in the mean values among treatments were considered significant at $P \leq 0.05$ and a trend towards significance at $0.05 < P < 0.10$, $n = 6$.
[2] Total AA = EAA + NEAA.

relative concentrations of taurine ($P < 0.001$), L-cysteine ($P = 0.004$), isoleucyl-lysine ($P < 0.001$), N-nervonoyl methionine ($P = 0.041$) and some other metabolites and decreased the plasma relative concentrations of glycocholic acid ($P = 0.013$), cholic acid ($P = 0.012$), chenodeoxycholic acid ($P = 0.012$) and some other metabolites.

Table 9 shows that compared with the control group, adding RPM increased the plasma relative concentrations of methionine ($P = 0.021$), L-isoleucine ($P = 0.036$), arginylisoleucine ($P = 0.024$), glycylphenylalanylleucylglycine ($P = 0.039$), N-acetylleucine ($P = 0.002$), L-tryptophan ($P = 0.006$), 4-hydroxy-L-proline ($P = 0.033$), and decreased the plasma relative concentrations of N-dodecylsarcosinate ($P = 0.029$), apigenin ($P = 0.035$) and carbenoxolone ($P = 0.005$). Table 10 indicates that compared with the RPM group, supplementing with RPT increased the plasma relative concentrations of taurine ($P = 0.015$), isoleucyl-lysine ($P = 0.008$), dimethylethanolamine ($P = 0.001$), N-nervonoyl methionine ($P = 0.006$) and some other metabolites, and decreased methionine ($P = 0.037$), S-lactoylglutathione ($P = 0.038$), arginylphenylalaninamide ($P = 0.007$), N-choloylglycine ($P = 0.008$).

3.7.2. Pathway analysis results

Fig. S4 shows that compared with the control group, RPT supplementation enriched five main metabolic pathways including taurine and hypotaurine metabolism, cysteine and methionine metabolism, steroid biosynthesis, primary bile acid biosynthesis and sulfur metabolism. Fig. S5 shows that compared with the control group, RPM supplementation enriched five main metabolic pathways including histidine metabolism, glycerophospholipid metabolism, pyrimidine metabolism, nucleotide metabolism and tyrosine metabolism.

Table 8
Differential plasma metabolites in steers fed RPT versus steers fed a control diet using a VIP threshold of 1.

Taxonomy super class	Metabolites	VIP[1]	FC[2]	*P*-value	Formula
Upregulated					
	Taurine	2.655	1.149	< 0.001	$C_2H_7NO_3S$
	L-Cysteine	1.874	1.056	0.004	$C_3H_7NO_2S$
	Isoleucyl-lysine	1.824	1.057	< 0.001	$C_{12}H_{25}N_3O_3$
	N-Nervonoyl methionine	1.060	1.027	0.041	$C_{29}H_{55}NO_3S$
	Bufotalin	1.131	1.019	0.020	$C_{26}H_{36}O_6$
	Acetohydroxamic acid	1.145	1.027	0.002	$C_2H_5NO_2$
	Cerebronic acid	1.524	1.032	0.043	$C_{24}H_{48}O_3$
	Carboprost	1.078	1.029	0.037	$C_{21}H_{36}O_5$
	Cholesteryl ferulate	1.821	1.052	< 0.001	$C_{37}H_{54}O_4$
	Cinobufagin	1.570	1.034	0.004	$C_{26}H_{34}O_6$
	Morpholine	1.171	1.029	0.018	C_4H_9NO
Downregulated					
	Glycocholic acid	2.147	0.932	0.013	$C_{26}H_{43}NO_6$
	Cholic acid	2.069	0.937	0.012	$C_{24}H_{40}O_5$
	Chenodeoxycholic acid	1.997	0.925	0.012	$C_{24}H_{40}O_4$
	Lithocholic acid glycine conjugate	2.620	0.899	0.001	$C_{26}H_{43}NO_4$
	3-Hydroxydodecanedioic acid	1.456	0.946	0.006	$C_{12}H_{22}O_5$
	Deoxycholic acid	2.021	0.931	0.015	$C_{24}H_{40}O_4$
	Chenodeoxyglycocholic acid	2.348	0.853	0.019	$C_{26}H_{43}NO_5$
	Oleamide	1.744	0.962	0.021	$C_{18}H_{35}NO$
	Deoxycytidine	2.273	0.872	0.019	$C_9H_{13}N_3O_4$
	Deoxycholic acid glycine conjugate	2.22	0.934	0.010	$C_{26}H_{43}NO_5$
	Panaxynol	2.768	0.823	0.011	$C_{17}H_{24}O$

RPT = rumen-protected taurine; VIP = variable importance in projection; FC = fold change.
$P \leq 0.05$ means significant difference between two groups.
[1] VIP refers to the contribution value of this metabolite to the difference between the two groups.
[2] FC refers to differential expression multiple of this metabolite between the two groups.

Table 9
Differential plasma metabolites in steers fed RPM versus steers fed a control diet using a VIP threshold of 1.

Taxonomy super class	Metabolites	VIP[1]	FC[2]	*P*-value	Formula
Upregulated					
	Methionine	1.562	1.022	0.021	$C_5H_{11}O_2NS$
	L-Isoleucine	1.116	1.010	0.036	$C_6H_{13}NO_2$
	Piperidine	1.156	1.012	0.027	$C_5H_{11}N$
	Aminomethyl fluorescein	2.157	1.038	0.030	$C_{21}H_{15}NO_5$
	(2S)-2-(4-Chloroanilinopropanoic acid	2.283	1.058	0.035	$C_9H_{10}ClNO_2$
	Indoleacrylic acid	1.163	1.009	0.008	$C_{11}H_9NO_2$
	Arginylisoleucine	1.268	1.019	0.024	$C_{12}H_{25}N_5O_3$
	2,5-Dihydroxybenzenesulfonic acid	1.398	1.017	0.028	$C_6H_6O_5S$
	Glycylphenylalanylleucylglycine	1.487	1.026	0.039	$C_{19}H_{28}N_4O_5$
	Ketoleucine	1.300	1.013	0.003	$C_6H_{10}O_3$
	N-Acetylleucine	1.838	1.038	0.002	$C_8H_{15}NO_3$
	5-Acetylamino-6-formylamino-3-methyluracil	1.196	1.013	0.005	$C_8H_{10}N_4O_4$
	4-Hydroxyphenylacetaldehyde	1.245	1.015	0.023	$C_8H_8O_2$
	Citramalic acid	1.028	1.012	0.040	$C_5H_8O_5$
	4-Hydroxy-L-proline	1.264	1.019	0.033	$C_5H_9NO_3$
	Allantoin	1.388	1.019	0.048	$C_4H_6N_4O_3$
	L-Tryptophan	1.513	1.018	0.006	$C_{11}H_{12}N_2O_2$
Downregulated					
	N-Dodecylsarcosinate	1.509	0.978	0.029	$C_{15}H_{31}NO_2$
	Apigenin	3.083	0.850	0.035	$C_{15}H_{10}O_5$
	Carbenoxolone	1.02	0.992	0.005	$C_{34}H_{50}O_7$
	(R)-Salsolinol	1.736	0.967	0.006	$C_{10}H_{13}NO_2$

RPM = rumen-protected methionine; VIP = variable importance in projection; FC = fold change.
$P \leq 0.05$ means significant difference between two groups.
[1] VIP refers to the contribution value of this metabolite to the difference between two groups.
[2] FC refers to differential expression multiple of this metabolite between two groups.

4. Discussion

4.1. Effects of RPM and RPT on rumen fermentation and nutrient digestibility

The *in vitro* rumen bypass rates of RPT and RPM which were determined using the two-step digestion technique of Tilley and Terry (1963) were 87.34% and 83.96%, respectively. The results of the present experiment showed that supplementing with RPT or RPM did not affect the pH, VFA, NH_3–N and MCP of rumen fluid. The results suggested that Met and taurine were well-protected from rumen microbial degradation.

Previous studies showed that supplementing with RPM at 8 g/d to early lactation cows fed with a 14.5% CP diet did not affect the apparent digestibility of DM, OM, N, NDF and ADF (Tamura et al., 2019) and supplementing with RPM at 15 g/d to lactating

Table 10
Differential plasma metabolites in steers fed RPT versus steers fed RPM using a VIP threshold of 1.

Taxonomy super class	Metabolites	VIP[1]	FC[2]	*P*-value	Formula
Upregulated					
	Taurine	2.027	1.136	0.015	$C_2H_7NO_3S$
	Isoleucyl-lysine	1.198	1.032	0.008	$C_{12}H_{25}N_3O_3$
	Dimethylethanolamine	1.492	1.056	0.001	$C_4H_{11}NO$
	N-Nervonoyl methionine	1.335	1.040	0.006	$C_{29}H_{55}NO_3S$
	Pubesenolide	2.703	1.121	0.001	$C_{28}H_{42}O_5$
	Leukotriene B4	2.373	1.152	0.006	$C_{20}H_{32}O_4$
	(S)-Alpha-terpinyl glucoside	1.450	1.047	0.021	$C_{16}H_{28}O_6$
	Cholesteryl ferulate	1.206	1.029	0.001	$C_{37}H_{54}O_4$
	N-Dodecylsarcosinate	1.268	1.035	0.014	$C_{15}H_{31}NO_2$
	2-O-(6-Phospho-alpha-mannosyl)-D-glycerate	1.119	1.039	0.037	$C_9H_{17}O_{12}P$
	Tetradecanedioic acid	1.726	1.064	0.023	$C_{14}H_{26}O_4$
	Senkyunolide N	1.327	1.030	0.010	$C_{12}H_{18}O_4$
	7alpha-Hydroxy-3-oxo-4-cholestenoate	1.319	1.036	0.003	$C_{27}H_{42}O_4$
	Isoketocamphoric acid	1.268	1.046	0.047	$C_{10}H_{16}O_5$
	9-Oxo-nonanoic acid	1.463	1.040	0.013	$C_9H_{16}O_3$
	Bufotalin	1.258	1.033	0.007	$C_{26}H_{36}O_6$
Downregulated					
	Methionine	1.224	0.970	0.037	$C_5H_{11}NO_2S$
	S-Lactoylglutathione	1.329	0.951	0.038	$C_{13}H_{21}N_3O_8S$
	Arginylphenylalaninamide	2.287	0.865	0.007	$C_{15}H_{24}N_6O_2$
	Deoxycholic acid	1.677	0.939	0.016	$C_{24}H_{40}O_4$
	Chenodeoxyglycocholic acid	2.327	0.844	0.013	$C_{26}H_{43}NO_5$
	N-Choloylglycine	1.028	0.974	0.008	$C_{26}H_{43}NO_6$
	Glycocholic acid	2.082	0.931	0.013	$C_{26}H_{43}NO_6$
	Cholylasparagine	2.060	0.862	0.035	$C_{28}H_{46}N_2O_7$
	Quercetin 3-O-sophoroside	1.943	0.903	0.026	$C_{14}H_{16}N_2O_6$
	Allodeoxycholic acid	2.480	0.875	0.026	$C_{24}H_{40}O_4$
	N-Phenylanthranilic acid	2.217	0.887	0.004	$C_{13}H_{11}NO_2$
	Dihydroneopterin phosphate	2.392	0.878	0.003	$C_9H_{14}N_5O_7P$
	Baptifoline	2.982	0.783	0.004	$C_{15}H_{20}N_2O_2$
	Methyleugenol	2.536	0.848	0.003	$C_{11}H_{14}O_2$
	7-Ketodeoxycholic acid	2.680	0.827	0.019	$C_{24}H_{38}O_5$
	Docosapentaenoic acid (22n-3)	2.626	0.828	0.019	$C_{22}H_{34}O_2$

RPT = rumen-protected taurine; RPM = rumen-protected methionine; VIP = variable importance in projection; FC = fold change.
$P \leq 0.05$ means significant difference between two groups.
[1] VIP refers to the contribution value of this metabolite to the difference between two groups.
[2] FC refers to differential expression multiple of this metabolite between two groups.

Holstein dairy cows fed with 17.3% CP and 16.4% diets did not affect the apparent digestibility of DM, CP, NDF, ADF or ash (Li et al., 2022). The results of the present experiment showed that supplementing with RPM did not affect the apparent digestibility of DM, OM, CP, NDF or ADF in steers. The results were in agreement with the previous studies.

It was reported that supplementing with unprotected taurine to beef steers up to 40 g/d did not affect the apparent digestibility of OM, CP or ADF (Liu et al., 2023), but linearly increased the NDF digestibility and tended to increase the apparent digestibility of DM. The present experiment showed that supplementing with RPT at 70.0 g/d (equivalent to 30.5 g/d metabolizable taurine) did not affect the digestibility of DM, OM, CP, NDF or ADF. The reason for the discrepancy in the results of the two experiments could be that unprotected taurine increased the relative abundance of ruminal cellulolytic bacteria in the experiment of Liu et al. (2023) whereas RPT directly flowed to the hindgut in the present experiment.

4.2. Effects of RPM and RPT on N metabolism

Methionine is an essential and limiting AA for cattle and can be directly used for protein synthesis. Previous experiments showed that supplementing with RPM at 9 g/d improved the milk production and the N utilization in lactating dairy cows fed a diet with 15.6% CP on a DM basis (Chen et al., 2011) and supplementing with RPM at 10, 20 and 30 g/d (equivalent to 16.5, 22.7, 28.8 g/d metabolizable Met) tended to increase the NR and the NUE in beef steers (Zhao et al., 2020). The present experiment showed that supplementing with RPM at 74.2 g/d (equivalent to 30.5 g/d metabolizable Met) decreased the urinary N excretion, and improved the NR and the NUE in beef steers. The results were in agreement with the experiments mentioned above. The reason for the decreased urinary N excretion and the increased NR and NUE induced by dietary supplementation of RPM could be that Met balanced the composition and the proportion of AA reaching the small intestine and increased the Met absorption (Sun et al., 2007). The results of the present experiment also showed that supplementing with RPT at 70.0 g/d (equivalent to 30.5 g/d taurine) had similar effects to RPM supplementation at 74.2 g/d Met (equivalent to 30.5 g/d metabolizable Met) on decreasing the urinary N excretion, and improving the NR and the NUE. The reason could be that supplementing with RPT reduced the amount of Met converted into taurine.

4.3. Effects of RPM and RPT on plasma AA and other biochemical indices

The results of the present experiment showed that supplementing with RPM increased the plasma concentration of total AA. The results were in agreement with Cantalapiedra-Hijar et al. (2020) who reported that supplementing with RPM at 7.0 g/d increased the plasma concentration of TAA in fattening Charolais bulls fed a diet containing 16.2% CP. The results of the present experiment also showed that supplementing with RPM increased the plasma concentrations of EAA as well as NEAA. Supplementing with RPM increased the plasma concentrations of Met, cysteine,

lysine, valine, isoleucine, leucine, asparagine, glycine and alanine. The reasons for these results are that Met can be transformed into cysteine in animal bodies (Wu, 2013) and deaminated to form oxaloacetate acid, and further transformed into asparagine, glycine and alanine (Wan et al., 2017). Supplementing with RPM at 74.2 g/d did not increase the plasma concentration of taurine. The results indicated that cysteine converted from Met was not further transformed into taurine.

The present experiment showed that RPT supplementation increased the plasma concentrations of NEAA and alanine, cysteine, serine, valine and glycine. The reason for the increased plasma concentration of alanine could be that taurine undergoes catabolism into alanine by pyruvate transaminase or α-Ketoglutarate transaminase in animal cells (Wu, 2020). The reason for the increased plasma concentration of cysteine could be that cysteine can be used as a precursor for taurine synthesis in animal body under the action of cysteine dioxygenase and cysteinesulfinate decarboxylase (Wen et al., 2019). RPT supplementation should have reduced the amount of cysteine for taurine synthesis. Another reason for the increased plasma concentration of cysteine could be that part of taurine was transformed into cysteine. However, this was not reported previously and it needs to be clarified in further research. The reasons for the increased plasma concentrations of serine, valine and glycine by RPT supplementation are unclear.

Leucine, isoleucine and valine which are branched-chain amino acid (BCAA) are able to stimulate muscle protein synthesis and also inhibit muscle protein degradation (Bolster et al., 2004; Escobar et al., 2006; Shimomura et al., 2006). Isoleucine not only serves as a substrate for protein synthesis but also plays a regulatory role in the pathways that regulate protein synthesis through the mammalian target of the rapamycin (mTOR) pathway (Xia et al., 2017). The present experiment showed that RPM supplementation increased the plasma concentrations of leucine, isoleucine and valine and RPT supplementation increased the plasma concentration of valine. The impacts of supplementing with RPM and RPT on the plasma AA are important reasons for the increased NR and NUE in beef steers.

4.4. Effects of RPM and RPT on urinary nitrogenous components

Urea accounts for the major part of urinary nitrogenous compounds (Dijkstra et al., 2013). The present experiment showed that supplementing with RPM decreased urinary urea excretion. The results were in agreement with Zhao et al. (2020) who reported similar effects of RPM supplementation in steers. The present experiment also showed that supplementing with RPT decreased the urinary excretions of N and urea. The results were similar to the impacts of RPM supplementation. The reason for the effects could be that supplementing with RPT reduced the amount of Met that could be converted into taurine.

Hippuric acid is another important nitrogenous compound in cattle urine. It is an acyl glycine formed in the liver by the conjugation of benzoic acid with glycine (Dijkstra et al., 2013). The results of the present experiment showed that supplementing with RPM or RPT increased the plasma concentration of glycine. This could be the reason for the increased urinary excretion of hippuric acid.

Animals are able to maintain the plasma concentration of taurine in a certain range (Ueki et al., 2012). The urinary excretion of taurine will be increased when taurine intake is high (Chesney et al., 1985). The present experiment showed that adding RPT increased the urinary excretion of taurine. The results suggested that the taurine in RPT was absorbed into blood and part of taurine in blood was excreted into urine. However, adding RPM did not increase the urinary excretion of taurine. The reason for the results could be that most part of the Met of RPM was utilized for body protein synthesis in steers and the increased level of taurine converted from Met did not reach a significant level.

Skeletal muscle is the main storage and metabolism site of AA and proteins in animal body and can reflect the body's protein metabolism (Kamei et al., 2020; Thalacker-Mercer et al., 2020). 3-MeHis is an AA existing in the skeletal muscle actin and myosin. It is produced after histidine is methylated in skeletal muscle protein synthesis in animal body and can be released from protein catabolism. The 3-MeHis released from protein catabolism cannot be used for protein synthesis but is quantitatively excreted into urine (Young et al., 1972). Hence, 3-MeHis is a suitable biomarker to identify skeletal muscle degradation (Kochlik et al., 2018). The present experiment indicated that supplementing with RPT or RPM decreased the urinary excretion of 3-MeHis compared with the control group and no difference in the urinary excretion of 3-MeHis between RPT and RPM groups. The results suggest that supplementing with RPT and RPM decreased the catabolism of skeletal muscle protein and the skeletal muscle protein degradation rate and taurine and Met have similar inhibitory impacts on skeletal muscle protein catabolism. The results were consistent with the improved NR, NUE and ADG by RPT and RPM supplementation.

4.5. Effects of RPM and RPT on plasma metabolomics

The plasma metabolomics profiling showed that supplementing with RPM upregulated the relative concentrations of plasma Met, L-isoleucine and L-tryptophan which are important for direct protein synthesis and some AA analogues such as N-acetyl-leucine, 4-hydroxy-L-proline and glycylphenylalanylleucylglycine, arginylisoleucine which are related to AA metabolism and protein synthesis. The metabolomics results were consistent with the results of plasma AA. Supplementing with RPM enriched the pathways of histidine metabolism and tyrosine metabolism which confirmed the effects of supplementing with RPM on the AA and protein metabolism in steers.

The metabolomics data of the present experiment showed that supplementing with RPT upregulated the plasma relative concentrations of taurine, L-cysteine, isoleucyl-lysine and N-nervonoyl methionine. The results were consistent with the plasma AA. Isoleucyl-lysine is a dipeptide composed of isoleucine and lysine and N-nervonoyl methionine is a nervonic acid amide of Met as well as an AA conjugate which is generated by acylamide conjugated to Met. The results indicated the links among taurine, Met and lysine. Supplementing with RPT enriched the pathways of taurine and hypotaurine metabolism, cysteine and methionine metabolism and sulfur metabolism. This may be an important link between taurine and Met, which could explain the reason why taurine and Met played similar roles in the N metabolism of steers.

The plasma metabolomics profiling showed that supplementing with RPM upregulated the plasma relative concentration of L-isoleucine while supplementing with RPT upregulated the plasma concentration of isoleucyl-lysine. The results were consistent with the plasma concentrations of AA which could also explain the impacts of RPT and RPM on improving the NR and the NUE in steers.

5. Conclusion

Supplementing with RPT and RPM showed similar impacts on improving the NR and the NUE in beef steers by regulating the plasma AA and some AA analogues. Supplementing with RPT spared part of Met for taurine synthesis in steer body. Whether taurine can be transformed into Met in the body of steers is unclear and needs to be investigated in the future.

Credit Author Statement

Yufeng Liu: Formal analysis, Investigation, Writing – original draft. **Cheng Liu:** Formal analysis, Investigation. **Shuo Zhang:** Formal analysis, Investigation. **Jinming Hu:** Formal analysis, Investigation. **Meng M. Li:** Data curation, Writing – review & editing. **Guangyong Zhao:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

Declaration of competing interest

We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, and there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the content of this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.aninu.2024.11.009.

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